

KARYOTYPES AND CASE STUDIES OF TEN INDIVIDUALS
WITH SUSPECTED SEX CHROMOSOME ANOMALIES

A Thesis
Presented to
The School of Graduate Studies
Drake University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by
Duane Dawson
August 1974

1974
D323

KARYOTYPES AND CASE STUDIES OF TEN INDIVIDUALS
WITH SUSPECTED SEX CHROMOSOME ANOMALIES

by
Duane Dawson

Approved by Committee:

Michael Myszewski

Chairman

Rodney Rogers

Joe Woods

~~Earle L. Canfield, Dean~~
Dean of the School of Graduate Studies

392088

KARYOTYPES AND CASE STUDIES OF TEN INDIVIDUALS
WITH SUSPECTED SEX CHROMOSOME ANOMALIES

An abstract of a Thesis by
Duane Dawson
August 1974
Drake University
Advisor: Michael E. Myszewski

The problem. This study will involve making a karyotype analysis and a case study of the residents of Woodward State Hospital and School who had been previously reported as having an abnormal X-chromatin constitution.

Procedure. Buccal smears were taken from each individual and stained with carbolfuchsin. Cells were observed for their X-chromatin content. Blood samples of each resident were drawn, cultured and harvested according to standard procedures. Chromosomes were counted in 30-50 cells and karyotypes prepared with emphasis on identifying the X-chromosome complement of each cell line. Case studies of the family history and physical features of each individual were done.

Findings. Six female residents suspected of having a 45,XO chromosome complement or possible 45,XO/46,XX mosaic condition were found to have a normal 46,XX chromosome complement. Two male residents suspected of having Turner's syndrome in the male were found to have a 46,XY chromosome complement. One male resident with a Klinefelter's phenotype had a 46,XY chromosome complement. One male had a 49,XXXXY chromosome complement.

Conclusions. The six females in the study were found not to have Turner's syndrome or mosaicism for the syndrome. Two males suspected of being male Turner's phenotypes were found to have Down's syndrome but no indication of Turner's syndrome. One male was found to have chromatin negative Klinefelter's syndrome. One male had a 49,XXXXY chromosome complement and showed all the phenotypic features of that condition.

TABLE OF CONTENTS

	PAGE
INTRODUCTION AND REVIEW OF THE LITERATURE	1
MATERIALS AND METHODS	14
RESULTS	18
DISCUSSION	26
LITERATURE CITED	35

LIST OF TABLES

TABLE	PAGE
1. Summary of findings in 10 cases of suspected X chromosome abnormalities.	27

INTRODUCTION AND REVIEW OF THE LITERATURE

"There is a diversity of errors of the sex chromosome complex in man because the X and Y chromosomes have certain properties that permit the survival of individuals with errors that would be lethal if they involved the non-sex chromosomes or autosomes." (Barr et al., 1969). For example, the presence in an individual of as many as five or six sex chromosomes is compatible with life although not normality, whereas such large excesses of any autosome has not been found (Barr et al., 1969).

Since 1959 a number of sex chromosome abnormalities have been observed in humans (Neu and Gardner, 1969). Techniques developed by Moorhead et al. (1960) have enabled researchers to karyotype chromosomes from peripheral blood leukocytes and identify numerical and structural abnormalities of the sex chromosomes. Rapid banding techniques where the chromosomes are treated with trypsin and then stained have shown specific banding patterns which have made possible the positive identification of individual chromosomes (Seabright, 1971). A standard system of chromosome nomenclature was established at the Paris Conference (1971). The system developed at this conference will be used throughout this paper.

There are two basic types of sex chromosome abnormalities: those which involve changes in the number of sex

chromosomes and those which involve structural changes. Nondisjunction is the mechanism responsible for changes in the chromosome number by causing unequal distribution of homologous chromosomes to daughter cells. If nondisjunction occurs in the female, both or no X chromosomes remain in the ovum; if it occurs in the male, sperm with both X and Y chromosomes or with neither chromosome may result.

Structural changes are the result of chromosome breakage. This may occur during the early stages of meiosis when breaking and rejoining of homologous chromosomes are taking place. If the broken ends fail to rejoin correctly, deletions, translocations and isochromosomes may result (Neu and Gardner, 1972). A deletion represents a loss of chromosome material. This occurs when the broken chromosomes fail to rejoin and leads to the loss of the chromosomal segment which does not contain the centromere. A translocation is the transfer of a section of one chromosome to a nonhomologous chromosome (Strickberger, 1969). An isochromosome is one which results from transverse rather than normal longitudinal division of the centromere and has two arms of identical length and homology (Neu and Gardner, 1972).

The results of sex chromatin studies usually give the first indication that an individual may have an error in the number of sex chromosomes present. The term sex chromatin denotes a body in the nucleus of a cell which shows a special relationship to the sex of the organism from which it came.

Sex chromatin can be used as a general term for either X or Y chromatin. In this discussion sex chromatin will be used to denote X-chromatin. Barr and Bertram (1949) described a distinction between neurons of male and female cats. A small body was present in the interphase nucleus of female neurons but absent in those of males (Mittwoch, 1967). Barr et al. (1950) showed sex chromatin to exist as a sex difference in human neurons. Moore and Barr (1954) found the same sex difference in many other tissues in humans. A significant finding by Davidson and Smith (1954) was that a sex difference could be demonstrated in leukocytes of peripheral blood. The sex specific body present in cells of females but not of males consists of a head attached to the body of the nucleus by means of a filament of varying length. The appendage is commonly called a "drumstick" and may be found on all types of polymorphonuclear leucocytes (PMN). The incidence of "drumsticks" in PMN's is about one per 38 neutrophils in females and none in males (Mittwoch, 1967). The sex chromatin test was further simplified when it was found that the presence or absence of sex chromatin could be demonstrated in cells scraped off the buccal mucosa (Moore and Barr, 1955).

When karyotyping techniques for human chromosomes became available in 1959, a correlation between the sex chromatin status and number of X chromosomes in an individual was established. The presence of sex chromatin was found to be associated with 2 or more X chromosomes in a nucleus

while its absence was found to be associated with the presence of a single X chromosome (Barr and Carr, 1960). Individuals found with 3 X chromosomes had 2 sex chromatin or Barr bodies and those with 4 X chromosomes had 3 Barr bodies.

It became clear that in individuals with normal as well as abnormal numbers of X chromosomes the maximum number of Barr bodies is one less than the number of X chromosomes present. Lyon (1961) formulated a hypothesis that the Barr body represented an X chromosome which was condensed and inactive. All X chromosomes in excess of one and the genes on them were inactive. Since the effect of this inactivation would be that animals of both sexes would have only a single dose of sex-linked genes, it is thought to provide the basis for dosage compensation in mammals (Lyon, 1963). In light of the interpretation made by Brown and Chandra (1973), the prediction of X-chromatin bodies is more accurate by using $S = X - A^m$, where A^m represents the number of maternal autosomal sets, S the expected number of sex-chromatin bodies and X the number of X chromosomes.

Sex chromatin studies became quite useful in detecting the classic XO type of Turner's syndrome, the XXY type of Klinefelter's syndrome and multiple-X conditions with or without Y chromosomes. Analysis which reveals cells with varying counts of Barr Bodies requires karyotyping to uncover possible sex chromosome mosaicism, the presence of 2 or more lines of cells with different numbers of X chromosomes in the

same individual.

There are four main types of aberrations of the sex chromosomes: Turner's syndrome, Klinefelter's syndrome, poly-X females and males with more than one Y chromosome. Turner's syndrome, Klinefelter's syndrome and poly-X females will be discussed in this review.

Turner's syndrome. Turner's syndrome bears the eponym of Turner who first described the conditions in 1938. It is a disorder of phenotypic females and is characterized by three basic features: short stature, sexual infantilism and "streak gonads" (fibrous tissue lacking follicles and ova). Often associated congenital malformations are lymphedema of the extremities at birth, broad shieldlike chest, webbed neck, cubitus valgus, coarction of the aorta, short fourth metacarpal, multiple pigmented nevi and a low hairline on the neck (Neu and Gardner, 1969).

Individuals with the syndrome are observed to be X-chromatin negative as first found independently by DeCourt et al. (1954), Polani et al. (1954), and Wilkins et al. (1954). The first published karyotype of a Turner's syndrome individual showed 45 chromosomes in the complement with one of the X chromosomes missing (Ford et al., 1959). The 45,X complement was confirmed by a number of investigators (Stewart, 1959; Tijo et al., 1959; Jacobs and Keay, 1959). This form of Turner's syndrome occurs approximately once in 3,000 female live births (Zellweger, 1968).

A number of individuals have what may be called variants of Turner's syndrome since their phenotypes fall within a spectrum with the classic XO condition at one extreme and normal XX females at the other. Short stature is common to all adult women in the XO group and most show one or more of the classic stigmata associated with the syndrome (Neu and Gardner, 1969). Although individuals with this complement are normally sterile, a case was reported by Bahner et al. (1960) where there was regular menstruation and the woman gave birth to a normal child. Another case was of a woman who had the XO condition and showed mild symptoms of the syndrome but gave birth to a normal 46,XX daughter. Buccal smears were negative and karyotypes from her peripheral blood consistently showed the 45,X chromosome complement (Nakashima and Robinson, 1971).

Individuals have been found who show symptoms of the syndrome with XO/XX mosaicism but the main features are less evident than in the XO group. One of 5 individuals with XO/XX mosaicism is in the normal height range and spontaneous menstruation is more common than in the XO group (Ferguson-Smith, 1965). Mikkelsen (1963) reported a case where normal ovaries were found. Carneiro Leao et al. (1966) demonstrated that nine pre-adolescent girls with XO/XX mosaicism presented varying degrees of the syndrome. The modified expression of the Turner's phenotype is thought to be due to the presence of the XX cell line (Ferguson-Smith, 1965).

Individuals with 45,X/46,XY mosaicism exhibit a variable phenotype. Short stature and other stigmata of Turner's syndrome may or may not be present. Gonads vary from streak gonads to infertile scrotal testes (Neu and Gardner, 1969). Cases of phenotypic females have been reported by Jacobs et al. (1961), Blank et al. (1963), and Judge et al. (1962) where the individuals display one or more Turner's syndrome symptoms. Ferrier et al. (1963) and Lewis et al. (1963) reported cases of 45,X/46,XY mosaicism where the individuals displayed a masculine phenotype but with varying degrees of testicular development and, in one case, a rudimentary Fallopian tube was found.

A case where the short arm of one X chromosome had been deleted (46,XXp-) giving it the appearance of a D group chromosome was reported by Jacobs et al. (1961). Others with the same cell line have been reported, all fulfilling the criteria for the diagnosis of Turner's syndrome (Neu et al., 1968).

Several cases have been reported where the long arm of one of the X chromosomes was considered deleted (46,XXq-) (Jacobs et al., 1960). The long arm deletion appears to be associated with streak gonads but does not appear to have a causative relation to the other phenotypic manifestations of Turner's syndrome (Neu and Gardner, 1969).

Individuals have been found with an isochromosome for the long arm of one X chromosome [46,X,i(Xq)]. Three such

cases with primary amenorrhea, poorly developed secondary sex characteristics and short neck were described by Fraccaro et al. (1960). These individuals had a positive buccal smear and the Barr body was larger than in normal XX females. They also showed other Turner's stigmata of short stature and streak gonads.

Lindsten and Tillinger (1962) reported a case of a human ring X chromosome. Other individuals reported since have been $45,X/46,XX_r$, $45,X/46,XX_r/46,X_rX_r$, and $45,X/46,XX/46,XX_r$ mosaics (Neu et al., 1968). These individuals showed spontaneous menstruation, diminished secondary sex characteristics and a low hairline. Their syndrome characteristics correspond most nearly to the $45,X/46,XX$ group (Neu and Gardner, 1969).

There are a few reports of males who have some of the developmental anomalies found in Turner's syndrome, in particular webbing of the neck and short stature. Frequently, prepubertal development is abnormal and some affected boys may show hypospadias and failure of one or both testes to descend. The testes are small, are abnormal histologically and have a failure of endocrine function. When such gonadal maldevelopment is found the eponym "Turner's syndrome in the male" is valid (Hamerton, 1971). Chromosome studies have been carried out on a number of these individuals (Steiker et al., 1961; Fraccaro et al., 1961; Polani, 1961) and a $46,XY$ normal male chromosome complement was found in all of them.

Ferguson-Smith (1965) has made some karyotype-phenotype correlations. All individuals with a missing short arm of one X chromosome show Turner's syndrome and are of short stature. Those cases with a missing long arm of one X chromosome show amenorrhea and streak gonads but not the short stature and other malformations usually found with the syndrome. Therefore, short stature and other Turner's symptoms must be produced by loss of the short arm of one X chromosome (Neu and Gardner, 1972).

Klinefelter's syndrome. Klinefelter et al. (1942) described nine phenotypic males characterized by gynecomastia, small testes with aspermatogenesis, an increased secretion of follicle-stimulating hormone (FSH) and a decreased excretion of 17-keto-steroids. A single sex chromatin body was found in a proportion of cells from such individuals fourteen years later (Bradbury et al., 1956; Plunkett and Barr, 1956). After studying the incidence of color-blindness in individuals with this syndrome, Polani et al. (1958) suggested that two X chromosomes may be present. In 1959, an individual with Klinefelter's syndrome was reported to have 47 chromosomes with an XXY sex chromosome complement (Jacobs and Strong, 1959).

Other symptoms often found associated with the syndrome are mental deficiency, sparse facial and body hair, feminine distribution of pubic hair, small phallus, small prostate gland and eunuchoidism (Neu and Gardner, 1969).

Most men with the syndrome are sterile although some have been reported to have offspring (Kaplan et al., 1963; Warburg, 1963). The testes at puberty are unable to respond normally to pituitary FSH and develop excesses of fibrous tissue, shrink and become aspermatogenic (Barr, 1966). Court Brown et al. (1964) found an incidence of sex chromatin positive males of 2.06 per 1,000 in newborns and an incidence of 9.71 per 1,000 in mental institutions. The extra X chromosome apparently increases the risk of mental retardation.

Variants of the basic 47,XXY syndrome have been described. The first case of an XXXY sex chromosome constitution was reported by Ferguson-Smith et al. (1960). These individuals show two sex chromatin bodies in their buccal smear cells. They are more severely retarded than 47,XXY males, have gross testicular changes and tend to be tall with long limbs (Neu and Gardner, 1969).

Fraccaro and Lindsten (1960) reported the first of many karyotypes describing 49,XXXXY individuals. Analysis of data on these cases shows that generally, the greater the number of X chromosomes present, the greater the degree of mental retardation. Often associated with the condition are hypogenitalism and delayed testicular descent. Skeletal anomalies including radio-ulnar stenosis are common. The symptoms of this condition are so characteristic of the XXXXY disorder that some prefer to think of it as a distinct

syndrome instead of classifying it as a variation of Klinefelter's syndrome (Barr, 1966).

Males with a 48,XXYY chromosome complement have been described (Muldal and Ockey, 1960; Schlegel et al., 1965; Ferguson-Smith, 1966). These individuals tend to be tall in stature and may have gynecomastia, acromegalic features and prognathism. They are similar to the 47,XXY cases but are generally more retarded and taller (Uchida et al., 1964).

A number of chromatin positive mosaics have been found and described. The 46,XY/47,XXY pattern is one of the most frequently observed karyotypes in Klinefelter's cases. The symptoms of Klinefelter's syndrome are usually less severe and often lacking. It appears that the normal 46,XY cell line masks the effect of the 47,XXY cell line (Ferguson-Smith, 1966). Other, more bizarre, mosaics, such as XX/XXY, showing varying degrees of the syndrome have been found but they are extremely rare (Neu and Gardner, 1969).

Approximately 25% of the males who are diagnosed as having Klinefelter's syndrome are sex chromatin negative and have an XY sex chromosome complement (Barr, 1966). They can have most of the same symptoms as the sex chromatin positive group including small testes and phallus, eunuchoidism and a feminine distribution of body hair, but they are generally less severely affected. They also show a lower incidence of gynecomastia. Diagnostically the two groups closely resemble each other. The major differences are histological; the sex

chromatin patterns and sex chromosome complements (Danowski, 1962).

Stewart (1959) found that between 0.1% and 0.2% of all phenotypic males are examples of chromatin positive Klinefelter's syndrome and that a lower percentage are of the chromatin negative type. He suggests that one or the other forms of Klinefelter's syndrome accounts for one-third of all cases of organic infertility in the male.

Poly-X females. Since 1959, a number of females with XXX sex chromosome complements have been reported. The individuals do not vary greatly from the normal female phenotype; most have regular menstrual periods, normal physical development and are fertile (Neu and Gardner, 1969). In some cases secondary amenorrhea and poorly developed secondary sex characteristics exist (Jacobs et al., 1961). About one-third of the triplo-X females have defects of a physical nature but since the majority are physically normal and the defects vary so greatly in type and severity, referring to the XXX condition as the Triplo-X syndrome may not be entirely correct (Barr et al., 1969).

Mental retardation aspects of the XXX condition are difficult to assess. The risk of mental retardation is undoubtedly high although a proportion of XXX females (the magnitude at present is unknown) are normal mentally (Barr et al., 1969). The frequency of triplo-X females in mental institutions varies from 6.72 per 1,000 (Fraser et al.,

1960) to 4.19 per 1,000 (Maclean et al., 1962). The frequency of the condition in a sample of newborn females was reported as 1.2 per 1,000 (Maclean et al., 1964).

Several cases of 48,XXXX females have been reported. All the individuals were physically normal with normal menstruation. They were all severely mentally retarded with IQ's of 50 or less (Hamerton, 1971). A 49,XXXXX girl was reported (Kesaree and Woolley, 1963) with more severe physical and mental retardation than the 47,XXX or 48,XXXX individuals.

Mosaics have been reported with 46,XX/47,XXX and 45,X/47,XXX cell lines (Maclean et al., 1962; Maclean et al., 1968; Court Brown et al., 1964). All were physically normal but had varying degrees of mental deficiency. Bergemann (1962) found individuals in the same family with 47,XXX/48,XXXX and 46,XX/47,XXX/48,XXXX mosaicism. No great deviation from the normal female phenotype was observed but mental deficiency was present.

It appears that the chance of mental deficiency increases as the number of X chromosomes increases. However, extra X chromosomes have in general a much less adverse affect on development, both physical and mental than does trisomy of any autosome (Barr et al., 1969).

The purpose of this investigation was to karyotype and make an individual case study of the residents of Woodward State Hospital and School who, as a result of a previous

study (Lyman, personal communication), were reported as having an abnormal X-chromatin makeup. Karyotypes were prepared with special emphasis on identifying the X chromosome complement of each individual. Case studies were made using information on file at the institution of the family history and physical features of each individual. Karyotype and phenotype correlations were made for each patient and compared with reports of these anomalies in the literature.

MATERIALS AND METHODS

Individuals were chosen for this study from reports by Lyman (personal communication) that 8 residents of Woodward State Hospital and School had shown unusual X-chromatin counts in buccal mucosa cells. Six of these residents were phenotypic females who should normally be Barr body positive but in Lyman's study showed a low incidence of X-chromatin bodies. They were karyotyped as a check for a possible Turner's syndrome (45,XO) chromosome makeup or for a possible mosaic condition (45,XO/46,XX). Two individuals were phenotypic males with abnormal X-chromatin counts. One was medically diagnosed as having Klinefelter's syndrome and should have been X-chromatin positive. Buccal smears showed a low incidence of X-chromatin bodies (Lyman, personal communication) and the individual was studied as a possible case of 46,XY sex chromatin negative Klinefelter's syndrome. The second male was chosen because he was reported to have

three cell lines: one line with no X-chromatin bodies, one line with one X-chromatin body and one line with three X-chromatin bodies (Lyman, personal communication). He was studied to determine his exact sex chromosome complement.

Two residents were suggested for study by hospital personnel as being possible Turner's syndrome males.

Buccal smears were made from each of the above individuals as a check on the previous reports of sex chromatin abnormalities. Cells for X-chromatin body analysis were collected by scraping the inside of one cheek with a standard wooden tongue depressor while pressing on the outside of the cheek with one hand. The material collected was then smeared as evenly as possible over a standard sized microscope slide and fixed by spraying once back and forth over the smear with "Spray-cyte" (Clay Adams). The smears were stained with carbolfuchsin. The staining technique involved immersing the slides in 0.5% celloidin solution for 45 seconds and air drying 30-45 seconds but not longer. The tissues were then hydrated by dipping the slides 10 times (1 second each time) in 80% methanol, 10 times in 70% methanol and 10 times in 50% methanol. After rinsing in tap water the slides were immersed in 5N HCl at room temperature for 20 minutes to remove cell cytoplasm and bacteria. The slides were rinsed 2-3 minutes in running tap water and stained in carbolfuchsin working solution for 10 minutes. The slides were then immersed in 95% methanol for 1 minute and absolute methanol

for 1 minute. The slides were then cleared with 10 dips in a mixture of 50% absolute methanol, 50% xylene and 10 dips in xylene. After drying, a cover glass was mounted on the slide with Euparal Vert. Cells were observed with the oil immersion lens (1250 X) of a Zeiss Photomicroscope II. Any darkly stained body near the periphery of the nuclear membrane of a cell was scored as a Barr body. The number of Barr bodies per cell was observed in at least 100 cells of each individual.

Ten ml of peripheral venous blood was drawn from each individual by a medical technologist at the hospital. The blood was placed in a heparinized (1 ml of solution containing 150 units sodium heparin) 10 ml culture tube, mixed by gentle inversion after stoppering and allowed to stand undisturbed for three hours. Two ml of the upper plasma layer containing leukocytes were drawn off into a sterile 2 ml pipette and placed in a 30 ml sterile culture flask (Falcon Plastics) with 8 ml of culture medium. The culture medium consisted of 85 ml of Minimal Essential Medium (Gibco), 15 ml of fetal calf serum (Gibco), 1 ml of penicillin/streptomycin (10,000 units/ml penicillin and 10,000 mcg/ml streptomycin, Gibco) and 1 ml of phytohemagglutinin (Gibco). One liter of medium was mixed under sterile conditions, divided into 100 ml bottles and frozen. One hundred ml amounts provided enough medium to prepare 12 culture flasks with 8 ml each and minimized breakdown of the medium from repeated thawing of a larger amount. After the culture flasks were inoculated,

they were incubated at 37°C ($\pm 0.5^{\circ}\text{C}$) for 68 hours. Two hours before harvest, 0.5 ml colcemid (Gibco) was added to give a final concentration of colcemid of 0.5 mcg/ml.

Harvesting techniques were modified from Moorhead et al. (1960). The culture medium and cells were swirled gently and poured into a conical graduated centrifuge tube, spun 12 minutes at 800 rpm and resuspended in 0.5 ml of the supernatant. With continual mixing, 3-4 ml of 0.075 M KCl (Gibco) hypotonic solution were added slowly. The mixture was incubated for 7 minutes at 37°C and centrifuged at 800 rpm for five minutes. The supernatant was quickly discarded, making the total time in the hypotonic solution 12 minutes. Three ml of the fixative solution (1:3 glacial acetic acid and absolute methanol at 37°C) were added and the cells were allowed to stand undisturbed for 30 minutes in the 37°C incubator. After centrifuging 10 minutes at 800 rpm, the supernatant was discarded and the cells were fixed twice for 5 minutes each time. The cells were suspended in 0.5 ml of fresh fixative for slide preparation.

Slides were prepared by dropping 4-5 drops of the cell suspension from a height of approximately 25 cm onto a chilled, wet slide. The slide was immediately passed through a flame to ignite the mixture and tilted in order to evenly distribute the suspension. When the flame went out, the slide was dried by blowing directly on the slide.

Staining was done by a technique modified from

Seabright (1971). The slides were placed in a solution of 0.001% trypsin (Gibco) and phosphate buffered saline (pH 7.2) for 75 seconds. They were immediately dehydrated in 50% methanol for 2 minutes followed by 100% methanol for 2 minutes. After rinsing in distilled water the cells were stained for 90 minutes in Gurr's Giemsa R66 stain (1 ml Gurr's Giemsa to 50 ml Gurr's buffer, pH 6.8). They were rinsed in distilled water and air dried.

Chromosome spreads suitable for counting were located with the low power objective lens (200 X) of a Zeiss Photomicroscope II. Chromosomes were counted under oil immersion (1250 X) in 30-50 metaphase cells and photographs were taken of 10-12 metaphase cells for karyotyping. Photographs were taken on Panatomic-X film (Kodak) and 5" x 7" prints were made on Kodabromide F-5 paper (Kodak). Karyotypes were made from cell lines which showed an abnormal chromosome number.

Case studies were obtained from information available in each resident's file at the hospital. Information concerning the physical and mental characteristics and the family history of each resident was recorded.

RESULTS

Resident number one was an 18 year old female, the third offspring in a family of six. She was the product of a 10 month pregnancy and three hours of labor. She appeared normal at birth. Both parents were in the 25-29 age range

when she was born. All other sibs were normal.

Her hospital diagnosis was, "Encephalopathy, convulsive disorder--abortive major motor seizures--genetic component undetermined". She was profoundly retarded with an IQ of less than 25. She showed impaired speech and responsiveness, was obese, ambulatory and could feed and dress herself.

Her buccal smears reveal a single X-chromatin body in 36% of her cells. Chromosome counts of 37 cells reveal 32 cells with 46 chromosomes and five cells with 45 chromosomes. Karyotypes show that her cells with 46 chromosomes have an XX sex chromosome complement and no unusual appearing chromosomes. Karyotypes of two cells with 45 chromosomes show a normal sex chromosome complement. One cell lacks a C group chromosome and the other lacks a G group chromosome. The other cells with 45 chromosomes are suitable only for chromosome counting and not for karyotyping because the chromosomes are unextended.

Resident number two was a 17 year old female, the first offspring in a family of four. The pregnancy was normal, with no complications or abnormal physical findings at birth. Both parents were in the 20-24 age range. The other sibs were normal but a maternal second cousin was reported to have mental deficiency.

Her hospital diagnosis was, "Mental retardation due to uncertain cause". She was classed as severely retarded

with an IQ of 25-39. She was slightly obese, had a speech defect and was reported as a hyperactive child by her parents.

Her buccal smears show 39% of her cells contain a single X-chromatin body. Chromosome counts show 31 cells with 46, one cell with 45 and one cell with 44 chromosomes. Karyotypes of a cell with 46 chromosomes show the normal sex chromosome complement. The cells with 45 and 44 chromosomes are not suitable for karyotyping.

Resident number three was a 22 year old female, born third in a family of five. Both the pregnancy and delivery were normal, but the child was small at birth and spent some time in an incubator. The mother had one miscarriage and one stillbirth. The other sibs were normal but there was mental deficiency in a maternal second cousin.

Her hospital diagnosis was "Mongolism". Her IQ was less than 50 and she was classed as severely retarded. She had a visual handicap and defective speech.

Buccal smear analysis reveals that 34% of her cells contain a single X-chromatin body. Chromosome counts reveal 33 cells with 47 chromosomes, five cells with 46 chromosomes and two cells with 45 chromosomes. Karyotypes show an extra G group chromosome and a normal XX sex chromosome complement in the cell line with 47 chromosomes. One of the five cells with 46 chromosomes has both a normal G group complement and an XX sex chromosome complement. One cell with 46 chromosomes lacks an F group chromosome and another lacks one of the A

group chromosomes but both have 5 G group chromosomes. Two of the cells with 46 chromosomes are not suitable for karyotyping. The cells with 45 chromosomes cannot be karyotyped.

Resident number four was a 23 year old female, the second child in a family of five. The pregnancy was normal but the mother reported she had fallen a few times. The delivery was also normal but the infant was described after delivery as most active, screaming and hyperkinetic. Both parents were in their mid-twenties at the time of the birth of the child. The other sibs were normal.

The hospital diagnosis was, "Encephalopathy due to prenatal injury (cerebral palsy due to prenatal anoxia)". She was classed as profoundly retarded with an IQ under 25. She did not speak, showed mild spasticity and paraplegia, and was restless, hyperactive and assaultive as a young child. She tended to be easily led and to prevent possible complications was sterilized at age 22.

Buccal smear analysis reveals that 32% of her cells contain a single X-chromatin body. Chromosome counts show 29 cells with 46 chromosomes and four cells with 45 chromosomes. Karyotypes show the cells with 46 chromosomes to have the normal XX sex chromosome complement. Karyotypes of two cells with 45 chromosomes show a normal XX sex chromosome complement. Chromosomes in the other two cells are suitable only for counting.

Resident number five was a 23 year old female, the

second born in a family of three. She was delivered by Caesarian section after a full, normal gestation. She had a small, misshapen head at birth. The other sibs were normal and there was no history of mental deficiency or epilepsy in the family.

The hospital diagnosis was, "Encephalopathy - convulsive disorder - abortive major motor seizures - genetic component undetermined". Her IQ was less than 25 and she was classed as profoundly retarded. She was short, muscular, hyperactive, aggressive at times and had a visual handicap. She had major motor seizures as a child and showed secondary microcephaly.

Buccal smear analysis reveals a single X-chromatin body in 34% of her cells. Chromosome analysis shows 33 cells with 46 chromosomes, two cells with 47 chromosomes and three cells with 45 chromosomes. Karyotypes reveal the normal XX sex chromosome complement in the cells with 46 chromosomes. The other cells are not suitable for karyotyping.

Resident number six was a 17 year old female, the product of a normal pregnancy, labor and birth. She was born fourth in a family of four. She was diagnosed at six months as having a "degenerative nerve disorder". An older child in the family had convulsions that were thought to be due to an auto accident. The child died after a craniotomy at Mayo Clinic. The brothers, sisters and mother of the

paternal family all had had febrile convulsions which have subsided.

The hospital diagnosis was, "Cerebral defect, congenital left ventricle". She was profoundly retarded with an IQ under 25. She had a hearing handicap and experienced major motor seizures, motor dysfunction, ataxia and spasticity.

Single X-chromatin bodies are apparent in 29% of her cells. Chromosome counts show 27 cells with 46 chromosomes and four cells with 45 chromosomes. Karyotypes reveal the normal XX sex chromosome complement in the cells with 46 chromosomes. Karyotypes of three of the cells with 45 chromosomes reveal a normal sex chromosome complement.

Resident number seven was a 21 year old male, the product of a normal pregnancy and birth. Genital anomalies were noted after birth. He was born second in a family of three. An older brother had been a resident of Woodward and had much the same condition, but a younger brother was normal.

His hospital diagnosis was, "Mental retardation due to uncertain cause". He was later diagnosed as having Klinefelter's syndrome. His IQ was under 25. He had a small penis located in a fold of tissue and had hypospadias. He had large pendulous breasts removed at age 17. He had a tendency to be overweight but was otherwise normal in appearance. He had been found to be sex chromatin negative.

Buccal smear analysis confirms the previous sex chromatin negative findings as no X-chromatin bodies were found in 100 cells counted. Chromosome counts reveal 30 cells with 46 chromosomes and three cells with 45 chromosomes. Karyotyping reveals a normal XY sex chromosome complement in the cells with 46 chromosomes. The cells with 45 chromosomes are not suitable for karyotyping.

Resident number eight was a 19 year old male. He was born prematurely after a 36 hour labor and a difficult breech delivery. He was reported as very weak after delivery. The other two sibs were normal and there was no history of retardation in the family. Both parents were in the 30-34 age range at the time of his birth.

The hospital diagnosis was, "Encephalopathy due to anoxemia at birth". He was profoundly retarded with an IQ of less than 25. There was no locomotion until the age of seven months. He had an undescended left testicle and poorly developed genitals. He showed hypertelorism and several bony defects. He had impaired motor and visual skills and did not speak. He was prone to much self abuse as evidenced by several facial scrapes and one apparently self-inflicted cauliflower ear.

Buccal smear analysis reveals that 29% of his cells contain three X-chromatin bodies, 1% contain two X-chromatin bodies and 4% contain a single X-chromatin body. Chromosome counts show 36 cells with 49 chromosomes, one cell with 48

chromosomes, one cell with 47 chromosomes and two cells with 46 chromosomes. Karyotyping reveals that the cells with 49 chromosomes have an XXXXY sex chromosome complement. The other cells contain small, unextended chromosomes and cannot be karyotyped.

Resident number nine was an 18 year old male, the product of a normal pregnancy and 13 hour labor. He was born fourth in a family of four. The other sibs were normal but the father had been treated for mental illness. The mother had had one miscarriage and was 39 years old at the time of the resident's birth.

The hospital diagnosis was, "Mongolism". He was profoundly retarded with an IQ of less than 25. He had impaired motor, auditory, visual and speech skills and was hyperactive. A paternal second cousin was diagnosed as having the same condition.

Barr body analysis reveals that none of 100 cells contain an X-chromatin body. Chromosome counts reveal 31 cells with 47 chromosomes and six cells with 46 chromosomes. Karyotypes of cells with 47 chromosomes reveal a trisomy condition in the G group chromosomes and an XY sex chromosome complement. Two cells with 46 chromosomes have the trisomy-G condition and XY sex chromosome complement but lack in one case a D group chromosome and in the other an F group chromosome. One cell with 46 chromosomes has the normal G group and XY sex chromosomes. The other three cells

with 46 chromosomes are suitable only for counting.

Resident number 10 was a 21 year old male, the product of a normal pregnancy and birth. He was third born in a family of three and was noted as having basic Mongoloid features at birth. Both parents were near 40 at the time of the resident's birth. He had a maternal second cousin who was diagnosed as Mongoloid.

His hospital diagnosis was, "Mongolism". His IQ was less than 25 and he was classed as profoundly retarded. He had a flattened occipital region and visual and speech handicaps.

Buccal smear analysis reveals that none of 100 cells contain an X-chromatin body. Chromosome counts show 28 cells with 47 chromosomes, two cells with 46 chromosomes and one cell with 45 chromosomes. Karyotypes reveal a trisomy condition in his G group chromosomes. The normal XY sex chromosome complement is present.

Table 1 shows a summary of X-chromatin analysis, chromosome counts and sex chromosome makeup for each individual included in this study.

DISCUSSION

Several surveys have been undertaken to ascertain the frequency with which sex chromosome abnormalities occur among the general population and among institutionalized individuals. The 45,X chromosome complement is found in 0.04% of

Table 1. Summary of findings in ten cases of suspected X-chromosome abnormalities.

Case Number	% with X-chromatin body (based on 100 cells)			Number of chromosomes						Total Cells Counted	Sex Chromosome Complement
	1	2	3	44	45	46	47	48	49		
Females											
1.	36	-	-	-	5(14%)	32(86%)	-	-	-	37	XX
2.	39	-	-	1(2%)	1(2%)	31(96%)	-	-	-	33	XX
3.	34	-	-	-	2(5%)	5(12%)	33(83%)	-	-	40	XX ⁺
4.	32	-	-	-	4(12%)	29(88%)	-	-	-	33	XX
5.	34	-	-	-	3(8%)	33(87%)	2(5%)	-	-	38	XX
6.	29	-	-	-	4(13%)	27(87%)	-	-	-	31	XX
Males											
7.	-	-	-	-	3(10%)	30(90%)	-	-	-	33	XY
8.	4	1	29	-	-	2(5%)	1(2.5%)	1(2.5%)	36(90%)	40	XXXXY*
9.	-	-	-	-	-	6(16%)	31(84%)	-	-	37	XY ⁺
10.	-	-	-	-	1(4%)	2(6%)	28(90%)	-	-	31	XY ⁺

+ Down's syndrome

* possible mosaic 46,XY/47,XXY/48,XXXY/49,XXXXY

newborn females (Maclean et al., 1964). There is evidence that many 45,X fetuses are lost in early pregnancy because up to 5.5% of aborted fetuses have had 45,X karyotypes (Carr, 1965). Kajii et al. (1973) found 7.89% (12 of 152) spontaneously aborted fetuses to be monosomic X. Maclean et al. (1962) found only one case of 45,X chromosome constitution among 4514 mental defectives in institutions but in the same study found chromatin positive males to occur with a frequency of 10.7 per 1000 individuals. In a study of newborns, the incidence of chromatin positive males was found to be about 2.6 per 1000 (Baikie et al., 1966). It appears that the degree of mental retardation is enhanced with any increase in the normal number of X chromosomes in a male or female. No figures for the incidence of the Turner's phenotype in the male have been compiled.

The six phenotypic females under consideration in the present study (residents 1, 2, 3, 4, 5 and 6) were chosen primarily because of a suspected mosaic condition of their sex chromosomes (45,X0/46,XX). Initial observations by Lyman (personal communication) indicated a low percentage of these individual's cells contained X-chromatin bodies. However, sex chromatin determinations with carbol-fuchsin staining in the present study revealed nothing abnormal about the number of their cells which contained X-chromatin bodies. A range of 29-39% of the cells counted in these individuals contained X-chromatin bodies. This is a high enough incidence of cells

with X-chromatin to consider the six residents definitely normal as far as X-chromatin is concerned (Mittwoch, 1967).

Karyotypes of these residents failed to show a mosaic condition of the sex chromosomes. A sex chromosome mosaic would show two or more cell lines with different numbers of X chromosomes. Resident number one showed five abnormal cells with 45 chromosomes but two of these were XX for their sex chromosome complement and lacked C and G group chromosomes. These chromosomes were probably lost during slide preparation. The other cells with 45 chromosomes showed small unextended chromosomes and could not be karyotyped. Residents two, four, five and six showed low percentages of abnormal cells with 45 chromosomes and could not be considered to be mosaic for their sex chromosomes.

Resident number three had been diagnosed as having Down's syndrome and karyotypes revealed her to have the classic trisomy-21 type of the syndrome. An individual with trisomy-21 and Turner's syndrome would have an XO sex chromosome constitution as well as three number 21 chromosomes for a total of 46 chromosomes. Chromosome counts revealed that 83% of her cells contained 47 chromosomes.

No pattern of defect could be found in the case histories of the six female residents except that all were severely (IQ less than 50) to profoundly (IQ less than 25) retarded and all showed varying types of physical handicaps. Resident number three showed enough symptoms of Down's

syndrome to be described in her hospital diagnosis as being a "mongoloid". The case histories and physical appearances of the others did not suggest Turner's syndrome or a mosaic condition for that syndrome. None of the classic Turner's syndrome symptoms was found except possibly in the case of resident number five who was reported as being "short". Cases of 45,XO/46,XX mosaicism reported (Ferguson-Smith, 1965; Mikkelsen, 1963; Carneiro Leao et al., 1966) have shown individuals with some symptoms of the syndrome but with features which are less evident than the classic 45,XO Turner's syndrome. One of five of these mosaic patients was in the normal height range; the others were considered short. Spontaneous menstruation was found to be more common than in the 45,XO group. One case was reported with normal ovaries. In every case however, some of the phenotypic features of Turner's syndrome were present.

When all results of this research were considered (X-chromatin results, karyotypes and case studies) it was concluded that none of the six females suspected of having Turner's syndrome (45,XO) or a mosaic condition for the syndrome (45,XO/46,XX) presented evidence which warranted that diagnosis. They all had normal numbers of cells with X-chromatin, their karyotypes revealed the XX sex chromosome complement to be present and none showed definite phenotypic features of Turner's syndrome.

Resident number seven had been suggested by Lyman

personal communication) as being X-chromatin negative and this was confirmed by the present research. While karyotypes revealed a normal XY sex chromosome complement, the case study revealed many symptoms of Klinefelter's syndrome. Rimoin et al. (1968) described a case of an individual with hypogonadism and gynecomastia who was found to have a normal male buccal smear and normal male karyotype. Rimoin et al. (1968) described another individual with gynecomastia, small testes and a small hypospadiac penis. This individual was also X-chromatin negative and had a normal male karyotype. Stewart et al. (1959) reviewed 32 cases of both chromatin positive and chromatin negative Klinefelter's syndrome. No great differences in phenotype were noted in the two groups as both groups showed classic Klinefelter's syndrome symptoms. One difference noted was that the chromatin positive group was taller and had disproportionately longer limbs than the chromatin negative group. The latter group was closer to normal in height and the limbs were not out of proportion. Resident number seven showed several symptoms of Klinefelter's syndrome (hypogonadism, gynecomastia and a hypospadiac penis). He was not above average in height and did not seem in outward appearance to be greatly abnormal. These phenotypic features match the classic Klinefelter's phenotype very closely. None of the cells of his buccal smear revealed X-chromatin and karyotypes revealed a 46,XY chromosome constitution. These conditions allow a conclusion that

resident number seven is a case of chromatin negative Klinefelter's syndrome.

Resident number eight presented a classic case of 49,XXXXY Klinefelter's syndrome. About 30 cases of this condition were described and summarized by Neu and Gardner (1969) and were found to usually show severe mental retardation, hypogonadism, radio-ulnar stenosis, hypertelorism, broad and flat nose, prognathism and large mouth. Tumba (1972) reviewed 70 cases of the XXXXY condition and found in addition to the previously mentioned characteristics that large, low set ears were usually present. He suggests that two sequential non-disjunctions of the X chromosome during maternal gametogenesis produce this condition and increased age of the mother seems to favor the anomaly. Resident number eight was reported in his hospital records to have all of these characteristics except the nose and mouth conditions which were evident upon visual examination when the blood sample was drawn. Counts of his chromosomes showed the predominant cell line to be 49,XXXXY but one cell with 48, one cell with 47 and two cells with 46 chromosomes were found. This suggests a possible mosaic condition, 46,XY/47,XXY/48,XXXY/49,XXXXY. Unfortunately the cells with 48, 47, and 46 chromosomes could not be karyotyped because the chromosomes were short and unextended. These cells could have been lacking chromosomes which were lost during slide preparation but the possibility of mosaicism was

reinforced by the sex chromatin results where cells with 0, 1, 2 and 3 X-chromatin bodies were found. Lyman (personal communication) found 22 cells with no Barr bodies, 49 cells with one Barr body, 28 cells with two Barr bodies and one cell with three Barr bodies in this resident. These results suggest mosaicism of the aforementioned type with predominant cell lines of the 47,XXY, 48,XXXY and 46,XY chromosome constitution. To positively diagnose a mosaic condition, more cells should be counted. Any cells with 46, 47 or 48 chromosomes should be karyotyped for their sex chromosome constitution.

The two suspected cases of a Turner phenotype in a male showed little to warrant this suspicion. Heller (1965) summarized the 43 reported cases of this condition and found them to usually show short stature, webbing of the neck and cubitus valgus and in some of the cases, mental retardation and poorly developed genitals. Heller's cases were sex chromatin negative males with a 46,XY chromosome complement. The two residents in this study were sex chromatin negative but both had a karyotype with an extra G group chromosome and an XY sex chromosome complement. They were both retarded and were reported in their hospital diagnosis to have the basic features of Down's syndrome. With the trisomy-21 condition and XY sex chromosome complements they would be expected to show cells with 47 chromosomes. Chromosome counts show them to have 47 chromosomes in their cells and

their karyotypes reveal a trisomy-21 condition. The only characteristic noted in these two residents which correlates with a male Turner's phenotype is the short stature noted for resident number nine. This alone is not enough to warrant the diagnosis of Turner's syndrome in a male.

The finding of no females at Woodward State Hospital with an abnormal number of X chromosomes seems unusual. Polani (1969) pooled data from several institutions studies and calculated that 45,XO karyotypes occur in one of 2574 institutionalized females and that sex chromosome mosaicism involving an XO cell line occurs with a frequency of one in 1402 institutionalized females. When the present study was being done, Woodward State Hospital had a female population of approximately 300 so it was not particularly unusual to find no 45,XO karyotypes or 45,XO/46,XX mosaics. What is unusual is that no 47,XXX karyotypes were found. Polani (1969) found that females with three X chromosomes occur in institutions with a frequency of one in 245.

Polani (1969) found chromatin positive males to occur with a frequency of one in 116 males in institutions. At Woodward State Hospital only one chromatin positive male was found in a population of approximately 250 males.

LITERATURE CITED

- Bahner, F., G. Schwarz, H. Heinz and K. Walter. 1960. Turner's syndrome with full secondary sex characteristics and fertility. *Acta Endocr. (Kbh)* 35:397.
- Baikie, A., M. Garson, S. Weste and J. Ferguson. 1966. Numerical abnormalities of the X chromosome. *Lancet* 1:398.
- Barr, M. 1966. The natural history of Klinefelter's syndrome. *Fert. and Steril.* 17:429.
- Barr, M. and E. Bertram. 1949. A morphological distinction between neurones of the male and female and behavior of nuclear satellites during accelerated nucleoprotein synthesis. *Nature* 163:676-77.
- Barr, M., L. Bertram and J. Lindsay. 1950. Morphology of the nerve cell nucleus, according to sex. *Anat. Record* 107:283-97.
- Barr, M. and D. Carr. 1960. Sex chromatin, sex chromosomes and sex anomalies. *Canad. Med. Assn. J.* 83:979.
- Barr, M., F. Sergovich, D. Carr and E. Shaver. 1969. The triplo-X female: An appraisal based on a study of 12 cases and a review of the literature. *Canad. Med. Assn. J.* 101:247-57.
- Bergemann, E. 1962. Family manifestations of the triplo-X karyotype: Preliminary communication. *J. Genet. Hum.* 10:370.
- Blank, C., D. Street and L. Segal. 1963. XO/XY mosaicism in a phenotypic female. *Lancet* 1:959.
- Bradbury, J., R. Bunge and R. Boccabella. 1956. Chromatin test in Klinefelter's syndrome. *J. Clin. Endocr.* 16:689.
- Brown, S. and S. Chandra. 1973. Inactivation system of the mammalian X chromosome. *Proc. Nat. Acad. Sci.* 1:195-99.
- Carneiro Leao, J., M. Voorhess, R. Schlegel and L. Gardner. 1966. XX/XO mosaicism in nine pre-adolescent girls with short stature as the presenting complaint. *Pediatrics* 38:972.
- Carr, D. 1965. Chromosome studies in spontaneous abortions. *Obstet. Gynec.* 26:308.

- Court Brown, W., D. Harnden, P. Jacobs, N. Maclean and D. Mantle. 1964. Abnormalities of the sex chromosome complement in man. Medical Research Council Special Report 305:21. Her Majesty's Stationery Office, London.
- Danowski, T. 1962. Clinical endocrinology, Vol. IV. Williams and Wilkins Co., Baltimore. 391p.
- Davidson, W., and D. Smith. 1954. Morphological sex difference in polymorphonuclear nuclear leucocytes. Brit. Med. J. 2:6-7.
- DeCourt, L., W. Sasso, E. Chiorboli and J. Fernandes. 1954. Sobre o sexo genetico nas pacientes com sindrome de Turner. Rev. Assn. med Brasil 1:203.
- Ferguson-Smith, M. 1965. Review article: Karyotype - phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. J. Med. Genet. 2:93.
- Ferguson-Smith, M. 1966. The sex chromatin. Edited by K. L. Moore. W. B. Saunders Co., Philadelphia. 396p.
- Ferguson-Smith, M., A. Johnston and A. Weinberg. 1960. The chromosome complement in true hermaphrodites. Lancet 2:126.
- Ferrier, P., S. Ferrier, D. Klein and C. Fernez. 1963. XX/XO mosaicism. Lancet 1:54.
- Ford, C., P. Polani, J. Briggs and P. Bishop. 1959. A presumptive human XXY/XX mosaic. Nature 183:1030.
- Fraccaro, M., D. Ikkos, J. Lindsten, R. Luft and K. Tillinger. 1961. Testicular germinal dysgenesis, male Turner's syndrome. Acta Endocrinol. 36:98.
- Fraccaro, M., K. Kaijser and J. Lindsten. 1960. Further cytological observations in gonadal dysgenesis. Ann. Hum. Genet. 24:205.
- Fraccaro, M. and J. Lindsten. 1960. A child with 49 chromosomes. Lancet 2:1303.
- Fraser, J., J. Campbell, R. MacGillivray, E. Boyd and B. Lennox. 1960. The XXX syndrome: Frequency among mental defectives and fertility. Lancet 2:626.
- Hamerton, J. 1971. Human cytogenetics, Vol. II. Academic Press, New York. 545p.

- Heller, R. 1965. The Turner phenotype in the male. *J. Pediatrics* 66:48-63.
- Jacobs, P., D. Harnden, K. Buckton, W. Court Brown, M. King, J. McBride, T. MacGregor and N. Maclean. 1961. Cytogenetic studies in primary amenorrhoea. *Lancet* 1:1183-88.
- Jacobs, P., D. Harnden, W. Court Brown, J. Goldstein, J. Close, T. MacGregor, N. Maclean and J. Strong. 1960. Abnormalities involving the X chromosome in women. *Lancet* 1:1213.
- Jacobs, P. and A. Keay. 1959. Somatic chromosomes in a child with Bonnevie-Ullrich syndrome. *Lancet* 2:732.
- Jacobs, P. and A. Strong. 1959. A case of human intersexuality having a possible XXY sex determining mechanism. *Nature* 183:302.
- Judge, M., M. Bodian, W. Cranston and P. Bowen. 1962. XO/XY mosaicism. *Cytogenetics* 1:104.
- Kajii, T., K. Ohama, N. Niikawa, A. Ferrier and S. Avirachan. 1973. Banding analysis of abnormal karyotypes in spontaneous abortion. *Am. J. Hum. Genet.* 25:539-547.
- Kaplan, J., M. Aspillaga, T. Shelley and L. Gardner. 1963. Possible fertility in Klinefelter's syndrome. *Lancet* 1:506.
- Kesaree, N. and P. Woolley. 1963. A phenotypic female with 49 chromosomes, presumably XXXXX. *J. Pediat.* 63:1099.
- Klinefelter, H., E. Reifstein and F. Albright. 1942. Syndrome characterized by gynecomastia, aspermatogenesis without a-leydigism and increased excretion of follicle stimulating hormone. *J. Clin. Endocr.* 2:615.
- Lewis, H., H. Nelson and J. Bowman. 1963. Mosaicism in Turner's syndrome, XO/XY. *Lancet* 1:352.
- Lindsten, J. and K. Tillinger. 1962. Self-perpetuating ring chromosome in a patient with gonadal dysgenesis. *Lancet* 1:593.
- Lyon, M. 1961. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* 190:372-3.
- Lyon, M. 1963. Attempts to test the inactive-X theory of dosage compensation in mammals. *Genet. Res.* 4:93-103.

- Maclean, N., D. Harnden, W. Court Brown, J. Bond and D. Mantle. 1964. Sex chromosome abnormalities in newborn babies. *Lancet* 1:286.
- Maclean, N., D. Harnden, J. Williams, D. Mantle and D. Jones. 1968. Abnormalities of sex chromosomes in man. *Lancet* 1:359-61.
- Maclean, N., J. Mitchell, D. Harnden, J. Williams, P. Jacobs, K. Buckton, A. Baikie, W. Court Brown, J. McBride, J. Strong, H. Jones and D. Jones. 1962. A survey of sex chromosome abnormalities among 4514 mental defectives. *Lancet* 1:293-298.
- Mikkelsen, M., A. Froland and J. Ellebjerg. 1963. XO/XX mosaicism in a pair of presumably monozygotic twins with different phenotypes. *Cytogenetics* 2:86.
- Mittwoch, U. 1967. Sex chromosomes. Academic Press, New York. 462p.
- Moore, K. and M. Barr. 1955. Smears from oral mucosa in detection of chromosomal sex. *Lancet* 2:57-58.
- Moore, K. and M. Barr. 1954. Nuclear morphology according to sex in human tissues. *Acta Anat.* 21:197-208.
- Moorhead, P., P. Nowell, W. Mellman, D. Battips and D. Hungerford. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* 20:613-616.
- Muldal, S. and C. Ockey. 1960. The double male - a new chromosome constitution in the Klinefelter's syndrome. *Lancet* 2:492.
- Nakashima, I. and A. Robinson. 1971. Fertility in a 45,X female. *Pediatrics* 47:770.
- Neu, R. and L. Gardner. 1969. Abnormalities of the sex chromosomes. Chap. 9. Endocrine and genetic diseases of childhood. Edited by L. I. Gardner. W. B. Saunders Co., Philadelphia. 695p.
- Neu, R. and L. Gardner. 1972. Clinical aspects of abnormalities of the X and Y chromosomes. *Clin. Obstet. Gyn.* 15:141-156.
- Neu, R., T. Kajii, T. Nolan and L. Gardner. 1968. 45,X/46,XXp- mosaicism in a prepubertal girl. *Ann. de Genet.* 11:11.

- Paris Conference (1971): Standardization in human cytogenetics. Birth defects; Original article series, VIII: 7,1972. The National Foundation, New York.
- Plunkett, E. and M. Barr. 1956. Testicular dysgenesis affecting the seminiferous tubules principally with chromatin positive nuclei. *Lancet* 2:853.
- Polani, P. 1961. Turner's syndrome and allied conditions. Clinical features and chromosome abnormalities. *Brit. Med. Bull.* 17:200-205.
- Polani, P. 1969. Abnormal sex chromosomes and mental disorders. *Nature (London)* 223:680-686.
- Polani, P., P. Bishop, B. Lennox, M. Ferguson-Smith, J. Stewart and A. Prader. 1958. Color vision studies and the X chromosome constitution of Klinefelter's syndrome patients. *Nature* 182:1092.
- Polani, P., W. Hunter and B. Lennox. 1954. Chromosomal sex in Turner's syndrome with coarctation of the aorta. *Lancet* 2:120.
- Rimoin, D., D. Borgoankar, S. Asper and R. Blizzard. 1968. Chromatin negative hypogonadism in phenotypic men. *Amer. J. Med.* 44:225.
- Seabright, M. 1971. A rapid banding technique for human chromosomes. *Lancet* 2:971-972.
- Schlegel, R., A. Aspillaga, R. Neu and L. Gardner. 1965. Studies on a boy with XYY chromosome constitution. *Pediatrics* 36:113.
- Steiker, D., W. Mellman, A. Bongiovanni, W. Eberlein and G. Leboeuf. 1961. Turner's syndrome in the male. *J. Pediat.* 58:321.
- Stewart, J. 1959. The chromosomes in man. *Lancet* 1:833.
- Stewart, J., W. Mack, A. Govan, M. Ferguson-Smith and B. Lennox. 1959. Klinefelter's syndrome, clinical and hormonal aspects. *Quart. J. Med.* 112:561.
- Strickberger, M. 1969. *Genetics*. The Macmillan Co., Toronto. 868p.
- Tijo, J., T. Puck and A. Robinson. 1959. The somatic chromosome constitution of some human subjects with genetic defects. *Proc. Nat. Acad. Sci. U.S.A.* 45: 1008.

- Tumba, A. 1972. The XXXXY phenotype: Analytical and synthetic study of 3 personal cases and 67 other cases from the literature. *J. Genet. Hum.* 20(1):9-48.
- Uchida, I., J. Miller and H. Solton. 1964. Dermatoglyphics associated with the XYY chromosome complement. *Am. J. Hum. Genet.* 16:284.
- Warburg, E. 1963. A fertile patient with Klinefelter's syndrome. *Acta Endocr.* 43:12.
- Wilkins, L., M. Grumbach and J. Van Wyk. 1954. Chromosomal sex in "Ovarian Agenesis". *J. Clin. Endocrin.* 14:1270.
- Zellweger, H. 1968. Human cytogenetics II. *J. Iowa Med. Soc.* 471-79.